

```

=> protein()c
    1049535 PROTEIN
    1715823 C
L1      5513 PROTEIN(W)C

=> assay or test
    201734 ASSAY
    413550 TEST
L2      595720 ASSAY OR TEST

=> clot or coagulation
    5539 CLOT
    56606 COAGULATION
L3      60619 CLOT OR COAGULATION

=> l1 and l2 and l3
L4      416 L1 AND L2 AND L3

=> l4 and zymogen
    2725 ZYMOGEN
L5      11 L4 AND ZYMOGEN

=> d iall 1-11

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L5  ANSWER 1 OF 11  CAPLUS  COPYRIGHT 1999 ACS
ACCESSION NUMBER:      1998:79061  CAPLUS
DOCUMENT NUMBER:      128:203449
TITLE:                Both cellular and soluble forms of thrombomodulin
                        inhibit fibrinolysis by potentiating the activation of
                        thrombin-activable fibrinolysis inhibitor
AUTHOR(S):            Bajzar, Laszlo; Nesheim, Michael; Morser, John; Tracy,
                        Paula B.
CORPORATE SOURCE:     Department of Biochemistry, University of Vermont
                        College of Medicine, Burlington, VT, 05405, USA
SOURCE:               J. Biol. Chem. (1998), 273(5), 2792-2798
                        CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER:            American Society for Biochemistry and Molecular
                        Biology
DOCUMENT TYPE:         Journal
LANGUAGE:              English
CLASSIFICATION:        13-5 (Mammalian Biochemistry)
ABSTRACT:

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Thrombin-activable fibrinolysis inhibitor (TAFI) is a recently described plasma \*\*\*zymogen\*\*\* that can be activated by thrombin to an enzyme with carboxypeptidase B-like activity. The enzyme, TAFIa, potentially attenuates fibrinolysis. TAFI activation, like \*\*\*protein\*\*\* \*\*\*C\*\*\* activation, is augmented about 1250-fold by thrombomodulin (TM). In this work, the effects of both sol. and cellular forms of TM on TAFI activation-dependent suppression of fibrinolysis were investigated. Sol. TM included in clots formed from purified components, barium citrate-adsorbed plasma, or normal human plasma maximally increased the tissue plasminogen activator-induced lysis time 2-3-fold, with satn. occurring at 5, 10, and 1 nM TM in the three resp. systems. Sol. TM did not effect lysis in the system of purified components lacking TAFI or in plasmas immunodepleted of TAFI. In addn., the antifibrinolytic effect of TM was negated by monoclonal antibodies against either TAFI or TM. The inhibition of fibrinolysis by cellular TM was assessed

by forming clots in dialyzed, barium citrate-adsorbed, or normal plasma over cultured human umbilical vein endothelial cells (HUVECs). Tissue plasminogen activator-induced lysis time was increased 2-fold, with both plasmas, in the presence of HUVECs. The antifibrinolytic effect of HUVECs was abolished 66% by specific anti-TAFI or anti-TM monoclonal antibodies. A newly developed functional \*\*\*assay\*\*\* demonstrated that HUVECs potentiate the thrombin-catalyzed, TM-dependent formation of activated TAFI. Thus, endothelial cell TM, in vitro at least, appears to participate in the regulation of not only \*\*\*coagulation\*\*\* but also fibrinolysis.

SUPPL. TERM: fibrinolysis inhibition thrombomodulin TAFI  
 INDEX TERM: Fibrinolysis  
 (both cellular and sol. forms of thrombomodulin inhibit fibrinolysis by potentiating the activation of thrombin-activable fibrinolysis inhibitor)  
 INDEX TERM: Thrombomodulin  
 ROLE: BAC (Biological activity or effector, except adverse); BIOL (Biological study)  
 (both cellular and sol. forms of thrombomodulin inhibit fibrinolysis by potentiating the activation of thrombin-activable fibrinolysis inhibitor)  
 INDEX TERM: 37329-68-3, TAFI  
 ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)  
 (both cellular and sol. forms of thrombomodulin inhibit fibrinolysis by potentiating the activation of thrombin-activable fibrinolysis inhibitor)

L5 ANSWER 2 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:24902 CAPLUS

DOCUMENT NUMBER: 128:113307

TITLE: \*\*\*Protein\*\*\* \*\*\*C\*\*\* activation and factor Va inactivation on human umbilical vein endothelial cells  
 AUTHOR(S): Hockin, Matthew F.; Kalafatis, Michael; Shatos, Marie; Mann, Kenneth G.

CORPORATE SOURCE: College of Medicine, Department of Biochemistry, University of Vermont, Burlington, VT, 05405-0068, USA  
 SOURCE: Arterioscler., Thromb., Vasc. Biol. (1997), 17(11), 2765-2775

CODEN: ATVBFA; ISSN: 1079-5642

PUBLISHER: American Heart Association

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 13-5 (Mammalian Biochemistry)

ABSTRACT:

The inactivation of factor Va was examd. on primary cultures of human umbilical vein endothelial cells (HUVECs), either after addn. of activated \*\*\*protein\*\*\* \*\*\*C\*\*\* (APC) or after addn. of .alpha.-thrombin and \*\*\*protein\*\*\* \*\*\*C\*\*\* (PC) \*\*\*zymogen\*\*\*. Factor Va proteolysis was visualized by Western blot anal. using a monoclonal antibody (.alpha.HVaHC No. 17) to the factor Va heavy chain (HC), and cofactor activity was followed both in a clotting \*\*\*assay\*\*\* using factor V-deficient plasma and by quantitation of prothrombinase function. APC generation was monitored using the substrate 6-(D-VPR)amino-1-naphthalenebutylsulfonamide (D-VPR-ANSNHC4H9), which permits quantitation of APC at 10 pmol/L. Addn. of APC (5 nmol/L) to an adherent HUVEC monolayer (3.5.times.105 cells per well) resulted in a 75%

inactivation of factor Va (20 nmol/L) within 10 min, with complete loss of cofactor activity within 2 h. Measurements of the rate of cleavage at Arg506 and Arg306 in the presence and absence of the HUVEC monolayer indicated that the APC-dependent cleavage of the factor Va HC at Arg506 was accelerated in the presence of HUVECs, while cleavage at Arg306 was dependent on the presence of the HUVEC surface. Factor Va inactivation proceeded with initial cleavage of the factor Va HC at Arg506, generating an Mr 75,000 species. Further proteolysis at Arg306 generated an Mr 30,000 product. When \*\*\*protein\*\*\* (0.5 .mu.mol/L), .alpha.-thrombin (1 nmol/L), and factor Va (20 nmol/L) were added to HUVECs an APC generation rate of 1.56.+-.0.11.times.10<sup>-14</sup> mol/min per cell was obsd. With APC generated in situ, cleavage at Arg506 on the HUVEC surface is followed by cleavage at Arg306, generating Mr 75,000 and Mr 30,000 fragments, resp. In addn., the appearance of two novel products derived from the factor Va HC are obsd. when thrombin is present on the HUVEC surface: the HC is processed through limited thrombin proteolysis to generate an Mr 97,000 fragment, which is further processed by APC to generate an Mr 43,000 fragment. NH2-terminal sequence anal. of the Mr 97,000 fragment revealed that the thrombin cleavage occurs in the COOH-terminus of the intact factor Va HC since both the intact HC as well as the Mr 97,000 fragment have the same sequence. Our data demonstrate that the inactivation of factor Va on the HUVEC surface, initiated either by APC addn. or PC activation, follows a mechanism whereby cleavage is obsd. first at Arg506 followed by a second cleavage at Arg306. The latter cleavage is dependent on the availability of the HUVEC surface. This mechanism of inactivation of factor Va is similar to that obsd. on synthetic phospholipid vesicles.

SUPPL. TERM: blood \*\*\*coagulation\*\*\* factor Va \*\*\*protein\*\*\*  
 \*\*\*C\*\*\* ; umbilical vein endothelium factor Va

INDEX TERM: Umbilical vein  
 (endothelium; \*\*\*protein\*\*\* \*\*\*C\*\*\* activation  
 and factor Va inactivation on human umbilical vein  
 endothelial cells)

INDEX TERM: Protein degradation  
 ( \*\*\*protein\*\*\* \*\*\*C\*\*\* activation and factor Va  
 inactivation on human umbilical vein endothelial cells)

INDEX TERM: Vascular endothelium  
 (umbilical vein; \*\*\*protein\*\*\* \*\*\*C\*\*\* activation  
 and factor Va inactivation on human umbilical vein  
 endothelial cells)

INDEX TERM: 74-79-3, Arginine, biological studies  
 ROLE: BPR (Biological process); BIOL (Biological study);  
 PROC (Process)  
 (inactivation of factor Va on HUVEC surface in relation  
 to cleavage at Arg506 followed by second cleavage at  
 Arg306)

INDEX TERM: 9002-05-5, Factor Xa  
 ROLE: BAC (Biological activity or effector, except adverse);  
 BIOL (Biological study)  
 (influence of factor Xa concn. in assessment of factor Va  
 activity)

INDEX TERM: 42617-41-4, Activated \*\*\*protein\*\*\* \*\*\*C\*\*\*  
 ROLE: BAC (Biological activity or effector, except adverse);  
 BIOL (Biological study)  
 ( \*\*\*protein\*\*\* \*\*\*C\*\*\* activation and factor Va  
 inactivation on human umbilical vein endothelial cells)

INDEX TERM: 65522-14-7, Factor Va  
 ROLE: BPR (Biological process); BIOL (Biological study);

PROC (Process)  
 ( \*\*\*protein\*\*\* \*\*\*C\*\*\* activation and factor Va  
 inactivation on human umbilical vein endothelial cells)  
 INDEX TERM: 9002-04-4, Thrombin  
 ROLE: BAC (Biological activity or effector, except adverse);  
 BIOL (Biological study)  
 (.alpha.-; \*\*\*protein\*\*\* \*\*\*C\*\*\* activation and  
 factor Va inactivation on human umbilical vein  
 endothelial cells)

L5 ANSWER 3 OF 11 CAPLUS COPYRIGHT 1999 ACS  
 ACCESSION NUMBER: 1997:58060 CAPLUS  
 DOCUMENT NUMBER: 126:168357  
 TITLE: Nonenzymic anticoagulant activity of the mutant serine  
 protease Ser360Ala-activated \*\*\*protein\*\*\*  
 \*\*\*C\*\*\* mediated by factor Va  
 AUTHOR(S): Gale, Andrew J.; Sun, Xi; Heeb, Mary J.; Griffin, John  
 H.  
 CORPORATE SOURCE: Departments of Molecular and Experimental Medicine and  
 of Vascular Biology, The Scripps Research Institute,  
 La Jolla, CA, 92037, USA  
 SOURCE: Protein Sci. (1997), 6(1), 132-140  
 CODEN: PRCIEI; ISSN: 0961-8368  
 PUBLISHER: Cambridge University Press  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 CLASSIFICATION: 7-5 (Enzymes)  
 ABSTRACT:  
 The human plasma serine protease, activated \*\*\*protein\*\*\* \*\*\*C\*\*\*  
 (APC), primarily exerts its anticoagulant function by proteolytic inactivation  
 of the blood \*\*\*coagulation\*\*\* cofactors Va and VIIIa. A recombinant  
 active site Ser 360 to Ala mutation of \*\*\*protein\*\*\* \*\*\*C\*\*\* was  
 prep'd., and the mutant protein was expressed in human 293 kidney cells and  
 purified. The activation peptide of the mutant \*\*\*protein\*\*\* \*\*\*C\*\*\*  
 \*\*\*zymogen\*\*\* was cleaved by a snake venom activator, Protac C, but the  
 "activated" S360A APC did not have amidolytic activity. However, it did  
 exhibit significant anticoagulant activity both in clotting assays and in a  
 purified protein \*\*\*assay\*\*\* system that measured prothrombinase activity.  
 The S360A APC was compared to plasma-derived and wild-type recombinant APC.  
 The anticoagulant activity of the mutant, but not native APC, was resistant to  
 diisopropyl fluorophosphate, whereas all APCs were inhibited by monoclonal  
 antibodies against APC. In contrast to native APC, S360A APC was not  
 inactivated by serine protease inhibitors in plasma and did not bind to the  
 highly reactive mutant protease inhibitor M358R .alpha.1 antitrypsin. Since  
 plasma serpins provide the major mechanism for inactivating APC in vivo, this  
 suggests that S360A APC would have a long half-life in vivo, with potential  
 therapeutic advantages. S360A APC rapidly inhibited factor Va in a nonenzymic  
 manner since it apparently did not proteolyze factor Va. These data suggest  
 that native APC may exhibit rapid nonenzymic anticoagulant activity followed by  
 enzymic irreversible proteolysis of factor Va. The results of clotting assays  
 and prothrombinase assays showed that S360A APC could not inhibit the variant  
 Gln 506-FVa compared with normal Arg 506-FVa, suggesting that the active site  
 of S360A APC binds to FVa at or near Arg 506.

SUPPL. TERM: \*\*\*protein\*\*\* \*\*\*C\*\*\* factor Va serine arginine  
 INDEX TERM: 56-45-1, Serine, biological studies  
 ROLE: BAC (Biological activity or effector, except adverse);

BPR (Biological process); BIOL (Biological study); PROC (Process)  
 (360 residue; nonenzymic anticoagulant activity of the mutant serine protease Ser360Ala-activated  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* mediated by factor Va)  
 INDEX TERM: 74-79-3, Arginine, biological studies  
 ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)  
 (506 residue; nonenzymic anticoagulant activity of the mutant serine protease Ser360Ala-activated  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* mediated by factor Va)  
 INDEX TERM: 37259-58-8, Serine proteinase 42617-41-4, Activated  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* 65522-14-7, Blood-  
 \*\*\*coagulation\*\*\* factor Va  
 ROLE: BAC (Biological activity or effector, except adverse); BPR (Biological process); BIOL (Biological study); PROC (Process)  
 (nonenzymic anticoagulant activity of the mutant serine protease Ser360Ala-activated \*\*\*protein\*\*\* \*\*\*C\*\*\* mediated by factor Va)

L5 ANSWER 4 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1994:74138 CAPLUS

DOCUMENT NUMBER: 120:74138

TITLE: Construction, Expression, and Properties of a Recombinant Chimeric Human \*\*\*Protein\*\*\* \*\*\*C\*\*\*

with Replacement of Its Growth Factor-like Domains by Those of Human \*\*\*Coagulation\*\*\* Factor IX  
 AUTHOR(S): Yu, Shiqin; Zhang, Li; Jhingan, Ashish; Christiansen, William T.; Castellino, Francis J.

CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, 46556, USA

SOURCE: Biochemistry (1994), 33(3), 823-31

CODEN: BICHAW; ISSN: 0006-2960

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 13-5 (Mammalian Biochemistry)

ABSTRACT:

The cDNA encoding a chimeric human \*\*\*protein\*\*\* \*\*\*C\*\*\* (PC), in which its EGF-like regions have been replaced with equiv. structures from human \*\*\*coagulation\*\*\* factor IX (fIX), was constructed and the gene product was expressed in human 293 cells. A mol. subpopulation of the recombinant chimeric protein (r-[PC/.DELTA.EGF-1,2/.del.fIXEGF-1,2]) was purified that contained the full complement (9 residues/mol) of .gamma.-carboxyglutamic acid (Gla). After conversion by thrombin to its activated form (r-[APC/.DELTA.EGF-1,2/.del.fIXEGF-1,2]), this latter enzyme was found to possess approx. 10% of the activity of wild-type recombinant APC (wtr-APC) in an APTT \*\*\*assay\*\*\*. In \*\*\*assay\*\*\* systems employing purified components, the activity of the mutant enzyme toward prothrombinase cofactor Va (fVa) and tenase cofactor VIII (fVIII) was approx. 30% and <10%, resp., of that of wtr-APC. The chimeric protein displayed full reactivity with a Ca2+-dependent monoclonal antibody to the Gla domain of PC, yielding a C50 for Ca2+ that was very similar to that obtained with wtr-PC (.apprx.3.7 mM). Titrns. of the dependency on Ca2+ of the intrinsic fluorescence of r-[PC/.DELTA.EGF-1,2/.del.fIXEGF-1,2] allowed calcn.

of a C50 value of 0.34 mM, again very similar to that of wtr-PC. As with wtr-PC, Ca<sup>2+</sup> inhibited the thrombin-catalyzed activation of r-[PC/.DELTA.EGF-1,2/.del.fIXEGF-1,2] with aKi of 148 .mu.M, as compared to a Ki of 125 .mu.M for wtr-PC. At a satg. level of Ca<sup>2+</sup>, activation of r-[PC/.DELTA.EGF-1,2/.del.fIXEGF-1,2] by the thrombin/thrombomodulin (thrombin/TM) complex occurred at approx. 70% of the rate of that of wtr-PC. The results suggest that (1) despite the substitution of substantial domain regions of the light chain of PC with those of a functionally unrelated protein, the chimeric protein retains essential features of PC \*\*\*zymogen\*\*\*; (2) the ability of PC to adopt its Ca<sup>2+</sup>-dependent conformation is not specifically dependent on its EGF-like regions; (3) the high-affinity Ca<sup>2+</sup> sites responsible for inhibition of the thrombin-catalyzed activation of PC, and stimulation of this same activation by thrombin/TM, are not specifically dependent on the EGF-like domains of PC; and (4) determinants present in the EGF-like domains of APC play a role in its anticoagulant properties, perhaps by directing specific alignments with its physiol. substrates on the phospholipid surface and/or through general subtle conformational properties of the enzyme that are dependent on the integrity of the EGF-like regions of PC. Addnl., the differences in activity of the mutant APC toward fVa and fVIII may be due to effects resulting from a specific interaction between the fIX EGF regions of [PC/.DELTA.EGF-1,2/.del.fIXEGF-1,2] and fVIII, a natural cofactor for fIXa.

SUPPL. TERM:       \*\*\*protein\*\*\*       \*\*\*C\*\*\*       EGFlke domain; blood  
                   \*\*\*coagulation\*\*\*       factor XIV EGF domain; chimera  
                   \*\*\*protein\*\*\*       \*\*\*C\*\*\*       \*\*\*coagulation\*\*\*       factor IX  
 INDEX TERM:       Blood       \*\*\*coagulation\*\*\*  
                   (inhibition of, by blood       \*\*\*coagulation\*\*\*       factor  
                   XIV, EGF-like domains role in)  
 INDEX TERM:       62229-50-9, EGF  
                   ROLE: BIOL (Biological study)  
                   (-like domains, of blood-       \*\*\*coagulation\*\*\*       factor  
                   XIV, functional properties dependent on)  
 INDEX TERM:       65522-14-7, Blood-       \*\*\*coagulation\*\*\*       factor Va  
                   ROLE: BIOL (Biological study)  
                   (blood-       \*\*\*coagulation\*\*\*       factor IX chimera with  
                   blood-       \*\*\*coagulation\*\*\*       XIV inactivation of)  
 INDEX TERM:       9001-27-8, Blood-       \*\*\*coagulation\*\*\*       factor VIII  
                   ROLE: BIOL (Biological study)  
                   (blood-       \*\*\*coagulation\*\*\*       factor IX chimera with  
                   blood-       \*\*\*coagulation\*\*\*       XIV inactivation of)  
 INDEX TERM:       60202-16-6,       \*\*\*Protein\*\*\*       \*\*\*C\*\*\*  
                   ROLE: BIOL (Biological study)  
                   (functional properties of, EGF-like domains role in)  
 INDEX TERM:       9001-28-9, Blood-       \*\*\*coagulation\*\*\*       factor IX  
                   ROLE: PRP (Properties)  
                   (growth factor-like domains of, replacement of EGF-like  
                   domains in blood-       \*\*\*coagulation\*\*\*       factor XIV by,  
                   functional properties modulation by)

L5   ANSWER 5 OF 11   CAPLUS   COPYRIGHT 1999 ACS

ACCESSION NUMBER:       1993:489590   CAPLUS

DOCUMENT NUMBER:       119:89590

TITLE:               A sensitive and facile       \*\*\*assay\*\*\*       for the  
                       measurement of activated       \*\*\*protein\*\*\*       \*\*\*C\*\*\*  
                       activity levels in vivo

AUTHOR(S):           Orthner, Carolyn L.; Kolen, Billy; Drohan, William N.

CORPORATE SOURCE:     Biomed. Res. Dev. Div., American Red Cross, Rockville,

MD, USA  
SOURCE: Thromb. Haemostasis (1993), 69(5), 441-7  
CODEN: THHADQ; ISSN: 0340-6245  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
CLASSIFICATION: 7-1 (Enzymes)

ABSTRACT:

Activated \*\*\*protein\*\*\* \*\*\*C\*\*\* (APC) is a serine protease which plays an important role as a naturally occurring antithrombotic enzyme. APC, which is formed by thrombin-catalyzed limited proteolysis of the \*\*\*zymogen\*\*\* \*\*\*protein\*\*\* \*\*\*C\*\*\*, functions as an anticoagulant by proteolytic inactivation of the \*\*\*coagulation\*\*\* cofactors VIIIa and Va. APC is inhibited by several members of the serpin family as well as by .alpha.2-macroglobulin. APC is being developed as a therapeutic for the prevention and treatment of thrombosis. An \*\*\*assay\*\*\* was developed to quantify circulating levels of enzymically active APC during its administration to patients, in healthy individuals, and in various disease states. This \*\*\*assay\*\*\* utilizes an EDTA-dependent anti- \*\*\*protein\*\*\* \*\*\*C\*\*\* monoclonal antibody (Mab) 7D7B10 to capture both APC and \*\*\*protein\*\*\* \*\*\*C\*\*\* from plasma, prepd. from blood collected in an anticoagulant supplemented with the reversible inhibitor p-aminobenzamidine. Mab 7D7B10-derivatized agarose beads are added to the wells of a 96-well filtration plate, equilibrated with Tris-buffered saline, and incubated for 19 min with 200 .mu.L of plasma. After washing, APC and \*\*\*protein\*\*\* \*\*\*C\*\*\* are eluted from the immunosorbent beads with a calcium-contg. buffer into the wells of a 96-well microtiter plate contg. antithrombin III (ATIII) and heparin. The amidolytic activity of APC is then measured on a kinetic plate reader following the addn. of L-pyroglutamyl-L-prolyl-L-arginine-p-nitroanilide (S-2366) substrate. The rate of substrate hydrolysis was proportional to APC concn. over a 200-fold concn. range (5.0 to 1,000 ng/mL) when measured continuously over a 15 to 30 min time period. The coeff. of variation was 5.9% at 35 ng/mL and 8.8% at 350 ng/mL APC. The sensitivity of the \*\*\*assay\*\*\* could be increased by measuring the amt. of color produced after longer incubation times in the endpoint mode. The measured APC activity levels were little affected by varying \*\*\*protein\*\*\* \*\*\*C\*\*\* or prothrombin over the extremes of 0 to 150% of normal plasma concns. By constructing the std. curve in \*\*\*protein\*\*\* \*\*\*C\*\*\* -deficient plasma, the concn. of APC activity in normal pooled plasma was detd. to be 2.8 ng/mL (45 pM), which represents 0.08% of the \*\*\*protein\*\*\* \*\*\*C\*\*\* concn. The \*\*\*assay\*\*\* was approx. 50-fold more sensitive than the identical \*\*\*assay\*\*\*, but using Mab-coated microtiter wells rather than immunosorbent beads as the capture step.

SUPPL. TERM: activated \*\*\*protein\*\*\* \*\*\*C\*\*\* immunoassay blood  
INDEX TERM: Antibodies  
ROLE: ANST (Analytical study)  
(monoclonal, to activated \*\*\*protein\*\*\* \*\*\*C\*\*\*  
in human blood, enzyme detn. in relation to)  
INDEX TERM: 42617-41-4, Activated \*\*\*protein\*\*\* \*\*\*C\*\*\*  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detn. of, in human blood, immunoassay for)

L5 ANSWER 6 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1991:57848 CAPLUS  
DOCUMENT NUMBER: 114:57848  
TITLE: Quantitative and functional assays compared for  
determination of \*\*\*zymogen\*\*\* and activated human  
\*\*\*protein\*\*\* \*\*\*C\*\*\*

AUTHOR(S): Richards, Susan M.; Olson, Timothy; Keyes, Lynne D.  
CORPORATE SOURCE: Genzyme Corp., Framingham, MA, 01701, USA  
SOURCE: Clin. Chem. (Winston-Salem, N. C.) (1990), 36(11),  
1892-6

CODEN: CLCHAU; ISSN: 0009-9147

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 7-1 (Enzymes)

Section cross-reference(s): 13

ABSTRACT:

Quant. and functional assays for \*\*\*protein\*\*\* \*\*\*C\*\*\*, using either purified \*\*\*protein\*\*\* \*\*\*C\*\*\* samples or pooled normal plasma as \*\*\*assay\*\*\* stds. were evaluated. The purified \*\*\*protein\*\*\* \*\*\*C\*\*\* samples were examd. as the \*\*\*zymogen\*\*\* form and after activation by thrombin. Mass concns. of \*\*\*protein\*\*\* \*\*\*C\*\*\* were detd. by amino acid anal. and confirmed by ELISA. Functional activity was assessed in both std. \*\*\*clot\*\*\* inhibition and amidolytic assays. The accuracy and precision of the ELISA was acceptable, with all 3 preps. of \*\*\*protein\*\*\* \*\*\*C\*\*\* having similar linear curves. The \*\*\*clot\*\*\* inhibition \*\*\*assay\*\*\* demonstrated marked variability when used according to the manufacturer's instructions; however, modifications to the protocol significantly decreased the CV, to <10%. Both activated \*\*\*protein\*\*\* \*\*\*C\*\*\* and the \*\*\*zymogen\*\*\* gave linear std. curves. Pooled normal human plasma gave a nonlinear curve, which contributed to inaccurate sample recoveries. The most nearly accurate recoveries were obtained when activated \*\*\*protein\*\*\* \*\*\*C\*\*\* was the \*\*\*assay\*\*\* std. Amidolytic assays provided no insights into the appropriateness of the preps. for that \*\*\*assay\*\*\* format. A uniform, consistent source of \*\*\*protein\*\*\* \*\*\*C\*\*\*, e.g., recombinant activated \*\*\*protein\*\*\* \*\*\*C\*\*\*, would be useful for standardizing all assays of \*\*\*protein\*\*\* \*\*\*C\*\*\*.

SUPPL. TERM: activated \*\*\*protein\*\*\* \*\*\*C\*\*\* detn blood

INDEX TERM: 42617-41-4, Activated \*\*\*protein\*\*\* \*\*\*C\*\*\*  
60202-16-6, Vitamin K-dependent \*\*\*protein\*\*\* \*\*\*C\*\*\*  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detn. of, in human blood plasma, quant. and functional  
assays for)

L5 ANSWER 7 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1990:547653 CAPLUS

DOCUMENT NUMBER: 113:147653

TITLE: Active site-specific immunoassays

AUTHOR(S): Mann, Kenneth G.; Williams, E. Brady; Krishnaswamy, Sriram; Church, William; Giles, Alan; Tracy, Russell P.

CORPORATE SOURCE: Dep. Biochem., Univ. Vermont, Burlington, VT, 05405, USA

SOURCE: Blood (1990), 76(4), 755-66

CODEN: BLOOAW; ISSN: 0006-4971

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 7-1 (Enzymes)

Section cross-reference(s): 15

ABSTRACT:

This study describes a process by which serine proteases that contain an S-1 arginine subsite and active site histidine may be inactivated and subsequently quantitated using a combination of peptidyl chloromethylketone chem. and immune



recognition technol. Active site labeling and inactivation of proteases is attained by modification of the active site histidine with a peptidyl chloromethylketone. In the specific illustrations demonstrated, the compd. biotinyl-.epsilon.-aminocaproyl-phenylalanylprolylarginyl chloromethylketone was used. This reagent reacts quant. and specifically with the active site histidine of a wide variety of proteases that are elaborated in the \*\*\*coagulation\*\*\* and fibrinolytic system. The inactivated enzyme(s) may be quantitated by combinations of antiprotein antibodies and avidin binding technol. using the biotin moiety on the peptide inhibitor. The capability of capture of inactivated enzyme products directly onto solid-phase avidin with subsequent quantitation of bound protein using specific antibodies has been demonstrated. In the converse system specific proteases were captured using antiprotein antibodies in the solid phase and bound enzyme quantitated by using avidin. Subsequent detection and quantitation has been achieved using the enzymic activity of horseradish peroxidase conjugated either to the antibody or to avidin. Both types of assays are feasible, with avidin capture being the preferred mode when enzyme is evaluated in the presence of excess \*\*\*zymogen\*\*\*, as would be common in the evaluation of most blood-clotting enzymes. Assays are illustrated for tissue plasminogen activator, plasmin, thrombin, factor Xa, and activated \*\*\*protein\*\*\* \*\*\*C\*\*\*, which can measure protease concns. as low as 50 pmol/L. Specific applications of the assays are provided in studies of the activation of prothrombin by the prothrombinase complex and of factor X with Russell's viper venom factor X activator. These assays measure the mass of active site present in the reaction mixt. and are relatively independent of subspecies of enzyme or the environment in which the activity is generated. These \*\*\*assay\*\*\* systems provide powerful tools for elucidating product-precursor relationships in multienzyme feedback reactions involving \*\*\*zymogen\*\*\* activation.

SUPPL. TERM: serine proteinase immunoassay blood \*\*\*coagulation\*\*\* ;  
 \*\*\*zymogen\*\*\* activation proteinase immunoassay blood  
 \*\*\*coagulation\*\*\*

INDEX TERM: Blood analysis  
 (plasminogen activator tissue-type recombinant form detn.  
 in, immunochem. methods for)

INDEX TERM: Immunochemical analysis  
 (immunoassay, serine proteinase zymogens and multiple  
 forms of human blood- \*\*\*coagulation\*\*\* system detn.  
 by)

INDEX TERM: 9001-26-7, Prothrombin  
 ROLE: BIOL (Biological study)  
 (activation of, of human, immunochem. methods for  
 detection of)

INDEX TERM: 9001-29-0, Blood- \*\*\*coagulation\*\*\* factor X  
 ROLE: BIOL (Biological study)  
 (blood- \*\*\*coagulation\*\*\* factor Xa of human detn. in  
 presence of, immunochem. detn. methods in relation to)

INDEX TERM: 9001-90-5, Plasmin 9002-04-4, Thrombin 9002-05-5, Blood-  
 \*\*\*coagulation\*\*\* factor Xa 42617-41-4, Activated  
 \*\*\*protein\*\*\* \*\*\*C\*\*\*  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (detn. of, of human, immunochem. methods for)

INDEX TERM: 37259-58-8, Serine proteinase  
 ROLE: BIOL (Biological study)  
 (multiple forms of, of blood- \*\*\*coagulation\*\*\* system  
 of human, immunochem. methods for detn. of)

INDEX TERM: 105913-11-9, Plasminogen activator

ROLE: BIOL (Biological study)  
(tissue-type, detn. of recombinant, in purified systems  
and in human plasma, immunochem. methods for)

L5 ANSWER 8 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1990:72942 CAPLUS  
DOCUMENT NUMBER: 112:72942  
TITLE: Snake \*\*\*protein\*\*\* \*\*\*C\*\*\* activator, methods  
of preparation and use thereof  
INVENTOR(S): Stocker, Kurt F.; Svendsen, Lars G.  
PATENT ASSIGNEE(S): Pentapharm A.-G., Switz.  
SOURCE: U.S., 11 pp.  
CODEN: USXXAM  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
INT. PATENT CLASSIF.:  
MAIN: A61K037-00  
US PATENT CLASSIF.: 514002000  
CLASSIFICATION: 7-3 (Enzymes)  
Section cross-reference(s): 1, 9, 12, 16  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4849403	A	19890718	US 1986-861786	19860509
AU 8657369	A1	19861204	AU 1986-57369	19860513
AU 605462	B2	19910117		
DK 8602248	A	19861130	DK 1986-2248	19860514
DK 165199	B	19921019		
DK 165199	C	19930301		
IL 78829	A1	19900831	IL 1986-78829	19860519
NO 8602118	A	19861201	NO 1986-2118	19860528
NO 166303	B	19910318		
NO 166303	C	19910626		
ES 555428	A1	19871201	ES 1986-555428	19860528
CA 1286223	A1	19910716	CA 1986-510137	19860528
JP 61280298	A2	19861210	JP 1986-122398	19860529
JP 07036760	B4	19950426		
ES 557670	A1	19880716	ES 1987-557670	19870814
ES 557670	A5	19880809		
PRIORITY APPLN. INFO.:			CH 1985-2267	19850529
			CH 1985-4135	19850925
			CH 1985-5087	19851128

OTHER SOURCE(S): MARPAT 112:72942

ABSTRACT:

A \*\*\*protein\*\*\* \*\*\*C\*\*\* activator is purified from the venom of Agkistrodon contortrix or from other snake venoms contg. immunol. cross reacting-material by chromatog. The activator is used to \*\*\*assay\*\*\* for \*\*\*protein\*\*\* \*\*\*C\*\*\*, to prevent or treat thrombotic disorders, and to obtain activated \*\*\*protein\*\*\* \*\*\*C\*\*\* from \*\*\*protein\*\*\* \*\*\*C\*\*\*

-contg. aq. media. The activator may also be obtained by culturing a recombinant microorganism contg. .gtoreq.1 gene for the activator. Chromogenic peptide substrates for measuring activated \*\*\*protein\*\*\* \*\*\*C\*\*\* are also described. A. contortrix venom was pretreated by dissolving it in H2O, adjusting the pH to 3.0, incubating the soln. at 70.degree. for 10 min, cooling

to 20.degree., adjusting the pH to 7.2, and centrifuging the resultant turbid soln. The residue was dissolved in H2O and chromatographed on DEAE-Sephadex A-50, CM-Sephadex C-50, and Sephadex G-100 to give pure \*\*\*protein\*\*\*  
 \*\*\*C\*\*\* activator. In a photometric \*\*\*assay\*\*\* of \*\*\*protein\*\*\*  
 \*\*\*C\*\*\*, human citrated plasma was incubated with the activator and activated  
 \*\*\*protein\*\*\* - \*\*\*C\*\*\* was detd. using 2AcOH.H-D-CHG-L-Pro-L-Arg-pNA (CHG  
 = cyclohexylglycine, pNA = p-nitroanilide) as chromogenic substrate and  
 measuring absorbance at 405 nm.

SUPPL. TERM:        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator Agkistrodon venom;  
                      antithrombotic        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator  
                      Agkistrodon; blood analysis        \*\*\*protein\*\*\*        \*\*\*C\*\*\*  
                      Agkistrodon activator; peptide substrate activated  
                      \*\*\*protein\*\*\*        \*\*\*C\*\*\*

INDEX TERM:        Microorganism  
                      (cloning in, of gene for        \*\*\*protein\*\*\*        \*\*\*C\*\*\*  
                      activator of Agkistrodon contortrix)

INDEX TERM:        Organ  
                      (exts.,        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        detn. in, activator  
                      from Agkistrodon contortrix venom for)

INDEX TERM:        Gene and Genetic element, animal  
                      ROLE: PROC (Process)  
                      (for        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator of  
                      Agkistrodon contortrix, cloning of)

INDEX TERM:        Molecular cloning  
                      (of gene for        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator of  
                      Agkistrodon contortrix)

INDEX TERM:        Agkistrodon contortrix  
                      Snake  
                      (        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator of venom of)

INDEX TERM:        Anticoagulants and Antithrombotics  
                      (        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator of Agkistrodon  
                      contortrix)

INDEX TERM:        Animal tissue culture  
                      (        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        detn. in, activator from  
                      Agkistrodon contortrix venom for)

INDEX TERM:        Venoms  
                      (snake,        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator of,  
                      purifn. of)

INDEX TERM:        Peptides, compounds  
                      ROLE: BIOL (Biological study)  
                      (conjugates, with chromogen, in        \*\*\*protein\*\*\*  
                      \*\*\*C\*\*\*        photometric detn. with activator from  
                      Agkistrodon contortrix venom)

INDEX TERM:        Peptides, compounds  
                      ROLE: BIOL (Biological study)  
                      (synthetic, conjugates, with chromogen, in  
                      \*\*\*protein\*\*\*        \*\*\*C\*\*\*        photometric detn. with  
                      activator from Agkistrodon contortrix venom)

INDEX TERM:        68987-32-6DP,        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        activator  
                      reaction products  
                      ROLE: PREP (Preparation)  
                      (activated        \*\*\*protein\*\*\*        \*\*\*C\*\*\*        manuf. from  
                      \*\*\*protein\*\*\*        \*\*\*C\*\*\*        with)

INDEX TERM:        98530-77-9  
                      ROLE: ANT (Analyte); ANST (Analytical study)  
                      (detn. of, activator from Agkistrodon contortrix venom)

for)  
INDEX TERM: 74-79-3, L-Arginine, biological studies  
ROLE: BIOL (Biological study)  
(di- or tripeptides contg. carboxy-terminal, in  
\*\*\*protein\*\*\* \*\*\*C\*\*\* detn. by activator from  
Agkistrodon contortrix venom)  
INDEX TERM: 72194-57-1 77672-32-3 88927-41-7 102565-94-6  
108963-65-1 108963-69-5  
ROLE: BIOL (Biological study)  
(in \*\*\*protein\*\*\* \*\*\*C\*\*\* photometric detn. with  
activator from Agkistrodon contortrix venom)  
INDEX TERM: 42617-41-4P, Activated \*\*\*protein\*\*\* \*\*\*C\*\*\*  
ROLE: PREP (Preparation)  
(prepn. of, from \*\*\*protein\*\*\* \*\*\*C\*\*\*  
\*\*\*zymogen\*\*\* , with activator from Agkistrodon  
contortrix venom)  
INDEX TERM: 9001-24-5, Blood- \*\*\*coagulation\*\*\* factor V 9001-27-8,  
Blood- \*\*\*coagulation\*\*\* factor VIII  
ROLE: BIOL (Biological study)  
( \*\*\*protein\*\*\* \*\*\*C\*\*\* detn. by activator from  
Agkistrodon contortrix venom in relation to)

L5 ANSWER 9 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1987:594480 CAPLUS  
DOCUMENT NUMBER: 107:194480  
TITLE: \*\*\*Assay\*\*\* methods for \*\*\*protein\*\*\*  
\*\*\*C\*\*\* (and protein S)  
AUTHOR(S): Nakamura, Satoko; Sakata, Yoichi  
CORPORATE SOURCE: Div. Hemostasis Thrombosis, Jichi Med. Sch.,  
Minami-Kawachi, Japan  
SOURCE: Rinsho Byori, Rinji Zokan (1987), (70), 100-7  
CODEN: RBRIAX; ISSN: 0370-3800  
DOCUMENT TYPE: Journal  
LANGUAGE: Japanese  
CLASSIFICATION: 9-10 (Biochemical Methods)  
Section cross-reference(s): 7

ABSTRACT:  
\*\*\*Assay\*\*\* methods for \*\*\*protein\*\*\* \*\*\*C\*\*\* and protein S are  
described. For each protein, immunol. methods, such as the Laurell method,  
2-dimensional immunoelectrophoresis, radioimmunoassay, and ELISA are  
applicable, and for \*\*\*protein\*\*\* \*\*\*C\*\*\* , which is a proteinase  
\*\*\*zymogen\*\*\* , chem. methods with synthetic substrate are also applicable.  
The clin. significance of congenital or acquired disorders of these proteins is  
discussed.

SUPPL. TERM: \*\*\*protein\*\*\* \*\*\*C\*\*\* S detn; blood  
\*\*\*coagulation\*\*\* factor XIV detn; vinectin detn  
INDEX TERM: Blood- \*\*\*coagulation\*\*\* factors  
ROLE: ANT (Analyte); ANST (Analytical study)  
(protein S, detn. of)  
INDEX TERM: 60202-16-6, Blood- \*\*\*coagulation\*\*\* factor XIV  
ROLE: ANT (Analyte); ANST (Analytical study)  
(detn. of)

L5 ANSWER 10 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1986:184356 CAPLUS  
DOCUMENT NUMBER: 104:184356

TITLE: \*\*\*Protein\*\*\* \*\*\*C\*\*\* levels in nephrotic syndrome: use of a new enzyme-linked immunoadsorbent \*\*\*assay\*\*\* for \*\*\*protein\*\*\* \*\*\*C\*\*\* antigen

AUTHOR(S): Soff, Gerald A.; Sica, Domenic A.; Marlar, Richard A.; Evans, Herbert J.; Qureshi, G. Dastgir

CORPORATE SOURCE: Dep. Med., Med. Coll. Virginia, Richmond, VA, USA

SOURCE: Am. J. Hematol. (1986), 22(1), 43-9  
CODEN: AJHEDD; ISSN: 0361-8609

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 14-12 (Mammalian Pathological Biochemistry)  
Section cross-reference(s): 7

ABSTRACT:

A competitive protein-binding ELISA for \*\*\*protein\*\*\* \*\*\*C\*\*\* was developed that was utilized to investigate if the hypercoagulability of the nephrotic syndrome is related to a deficiency of circulating plasma \*\*\*protein\*\*\* \*\*\*C\*\*\*. \*\*\*Protein\*\*\* \*\*\*C\*\*\* was measured in plasma of patients with nephrotic syndrome (24-h protein 8.4 g; serum creatinine 4.2 mg/dL). Azotemic nonnephrotic patients were employed as controls (serum creatinine 6.0 mg/dL). No significant redn. of \*\*\*protein\*\*\*

\*\*\*C\*\*\* values was obsd. (mean 108%, ranges 65-200%) in nephrotic patients when compared with the controls (mean 127%, range 100-200%), even though \*\*\*protein\*\*\* \*\*\*C\*\*\* antigen was present in all nephrotic urine samples tested. Also, no correlation existed between blood levels of urea N, creatinine, albumin, total protein, or 24-h urine protein excretion and the obsd. \*\*\*protein\*\*\* \*\*\*C\*\*\* values. Apparently, in patients with the nephrotic syndrome, a hypercoagulable state may not be related to a deficiency of \*\*\*protein\*\*\* \*\*\*C\*\*\*, and the level of this \*\*\*zymogen\*\*\* in nephrotic syndrome reflects a dynamic balance between urinary losses and systemic prodn.

SUPPL. TERM: \*\*\*protein\*\*\* \*\*\*C\*\*\* ELISA nephrosis; blood factor XIV ELISA nephrosis

INDEX TERM: Urine  
(compn. of, in nephrotic syndrome in humans, blood-\*\*\*coagulation\*\*\* factor XIV in relation to)

INDEX TERM: Kidney, disease or disorder  
(nephrotic syndrome, blood-\*\*\*coagulation\*\*\* factor XIV of humans in, ELISA in relation to)

INDEX TERM: 9000-94-6  
ROLE: BIOL (Biological study)  
(III, in nephrotic syndrome in humans, blood-\*\*\*coagulation\*\*\* factor XIV in relation to)

INDEX TERM: 60202-16-6  
ROLE: BIOL (Biological study)  
(in nephrotic syndrome in humans, detn. by ELISA in relation to)

INDEX TERM: 9001-27-8  
ROLE: BIOL (Biological study)  
(in nephrotic syndrome, in humans, blood-\*\*\*coagulation\*\*\* factor XIV in relation to)

L5 ANSWER 11 OF 11 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1984:170454 CAPLUS

DOCUMENT NUMBER: 100:170454

TITLE: The use of a functional and immunologic \*\*\*assay\*\*\*  
 for plasma \*\*\*protein\*\*\* \*\*\*C\*\*\* in the study  
 of the heterogeneity of congenital \*\*\*protein\*\*\*  
 \*\*\*C\*\*\* deficiency

AUTHOR(S): Bertina, R. M.; Broekmans, A. W.; Krommenhoek-van Es,  
 C.; Van Wijngaarden, A.

CORPORATE SOURCE: Dep. Intern. Med., Leiden Univ. Hosp., Leiden, Neth.

SOURCE: Thromb. Haemostasis (1984), 51(1), 1-5  
 CODEN: THHADQ; ISSN: 0340-6245

DOCUMENT TYPE: Journal

LANGUAGE: English

CLASSIFICATION: 7-1 (Enzymes)  
 Section cross-reference(s): 14

ABSTRACT:

\*\*\*Protein\*\*\* \*\*\*C\*\*\* is a vitamin K-dependent \*\*\*zymogen\*\*\* of a  
 serine proteinase, which is involved in blood \*\*\*coagulation\*\*\*. A  
 congenital deficiency in \*\*\*protein\*\*\* \*\*\*C\*\*\* antigen, which is  
 inherited as an autosomal dominant disorder, has been reported to be assocd.  
 with a high risk for thrombo-embolic disease at relatively young age. In the  
 present paper, the development of a functional \*\*\*assay\*\*\* for plasma  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* is reported. In this \*\*\*assay\*\*\*,  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* is adsorbed to Al(OH)<sub>3</sub>, eluted and activated by  
 thrombin, after which the concn. of the activated \*\*\*protein\*\*\* \*\*\*C\*\*\*  
 is measured with a peptide substrate (S 2366). Normal values for  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* activity and \*\*\*protein\*\*\* \*\*\*C\*\*\* antigen  
 were detd. in healthy volunteers and patients on stable oral anticoagulant  
 treatment. \*\*\*Protein\*\*\* \*\*\*C\*\*\* activity and antigen levels were  
 compared in 28 patients from 9 different pedigrees with both congenital  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* deficiency and thrombotic disease. Two types of  
 \*\*\*protein\*\*\* \*\*\*C\*\*\* deficiency could be recognized; in type I, the  
 deficiency is due to the absence or reduced presence of \*\*\*protein\*\*\*  
 \*\*\*C\*\*\* mols., whereas in type II, the deficiency is caused by the presence  
 of an abnormal \*\*\*protein\*\*\* \*\*\*C\*\*\* mol. with strongly reduced  
 functional activity.

SUPPL. TERM: \*\*\*protein\*\*\* \*\*\*C\*\*\* detn plasma genetic deficiency

INDEX TERM: Blood analysis  
 ( \*\*\*protein\*\*\* \*\*\*C\*\*\* detn. in, congenital  
 deficiency in relation to)

INDEX TERM: Proteins  
 ROLE: ANT (Analyte); ANST (Analytical study)  
 (C, detn. of, in blood plasma, congenital deficiency in  
 relation to)